



July 2, 2003

Joseph J. Merenda
Director, Office of Science Policy and Coordination
Office of Pollution Prevention and Toxic Substances
USEPA Headquarters, 7201
Ariel Rios Building
1200 Pennsylvania Avenue, N. W.
Washington, DC 20460

Re: Docket Control Number OPPTS-2003-0016
Issues Pertaining to EDMVS – Aromatase Assay

Dear Dr. Merenda,

The American Chemistry Council (ACC or the “Council”) has played an active role in the development and implementation of the endocrine disruptor screening and testing program (EDSP) for several years.¹ The Council supports the Agency’s establishment of the Endocrine Disruptor Methods Validation Subcommittee (EDMVS) to provide technical advice and recommendations to EPA concerning the validation of endocrine disruptor screening and testing methods. ACC looks forward to the timely development and implementation of a scientifically sound EDSP.

The Council represents more than 90 percent of the productive capacity for basic industrial chemicals within the United States and its members are the leading companies engaged in the business of chemistry. EPA’s endocrine disruptor screening and testing program (EDSP) may significantly affect the Council and its members. For that reason, the Council and its members have attempted to assist the Agency in developing and implementing its EDSP.

At the June 5-6, 2003 EDMVS meeting, EPA presented their laboratory results pertaining to standardization and validation of the aromatase assay. While EPA has made progress in

¹ The American Chemistry Council represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and safety performance through Responsible Care, common sense advocacy designed to address major public policy issues, and health and environmental research and product testing. The business of chemistry is a \$460 billion enterprise and a key element of the nation's economy. It is the nation’s largest exporter, accounting for ten cents out of every dollar in U.S. exports. Chemistry companies invest more in research and development than any other business sector. Safety and security have always been primary concerns of ACC members, and they have intensified their efforts, working closely with government agencies to improve security and to defend against any threat to the nation’s critical infrastructure.

standardizing the assay, the Council believes that EPA still needs to address some critical aspects of the assay prior to initiating a large-scale validation effort. First and foremost, EPA needs to be reminded that the desired EDSTAC objective was to develop either an *in vitro* or *in vivo* assay(s) with the ability to detect weak aromatase inhibitors. EPA seems to have focused on an *in vitro* approach, and should acknowledge that the Intact Male *in vivo* screening assay is an effective method too.

EPA proposes to embark on an extensive and costly validation effort for the human placental aromatase assay, and yet the Agency has not adequately addressed the overarching question: what actions would be triggered by a positive *in vitro* result in Tier 1 aromatase assay? The Agency needs to articulate how the results would be used because this impacts on the validation efforts. A test method must be validated for an intended purpose. It is insufficient for EPA to state that the purpose of the assay is to be used as a Tier 1 screen in the EDSP. In this regard, the Council believes that it would be inappropriate to jump to a Tier 2 mammalian reproduction test based solely on a positive *in vitro* aromatase result. Therefore, the Council recommends an *in vivo* confirmation of inhibitory activity, using the Intact Male assay, a mechanistic assay that satisfies the criteria for a Tier 1 screening method.

EPA appears to be focused on human placenta as the basis for the assay. There are a number of problems that laboratories face when using human tissues, and EPA has not adequately evaluated alternatives that do not have such attendant issues. For example, all labs conducting this assay will be required to undergo review and approval by an Institutional Review Board, and must conform to exacting requirements in accordance with Federal policies for the Protection of Human Subjects. Human placenta cannot be purchased commercially, and therefore each laboratory considering this assay would need to establish some means of obtaining tissues, which may be a significant burden. Furthermore, human tissues pose a significant risk to laboratory personnel for transmission of infections such as hepatitis and AIDS, and therefore consideration of infection control procedures and laboratory worker protection/liability issues can impact a given laboratory's decision as to whether to conduct or not conduct such assays.

In Vitro assays based on the recombinant enzyme CYP 19 (aromatase) would overcome a number of the disadvantages posed by the human placental aromatase method. However, use of the recombinant enzyme for commercial (testing) purposes is likely governed by patent restrictions. Therefore, before such methods could be used in commercial settings, the patent issues would need to be addressed. Since it is the intent of EPA to have the EDSP assays run in commercial facilities, before the recombinant protein aromatase assay could be considered 'validated' EPA will need to address such use issues.

As was discussed at the EDMVS meeting, EPA should not design these screening studies to be any more complex than is necessary to develop reliable data that is specific to address the needed decision. Therefore, EPA should consider a 2-step approach, where the initial step provides a 'yes' or 'no' answer as to whether aromatase inhibitory activity is detected. If the

answer is 'no,' then no additional work would be needed. If the answer is 'yes', then it may be appropriate to conduct further investigations to define the K_i , the inhibition constant for the rate of catalysis. It would be totally inappropriate to develop the assay protocol focused on defining K_i for all substances, since there is significant additional work to define K_i , and many, if not most, of the substances which will be evaluated are likely not to have specific aromatase inhibitory activity.

Finally, as part of the validation effort, EPA needs to develop guidance criteria on dose setting and interpreting data. All too often, *in vitro* assays are conducted with unrealistically high concentrations of test materials. This should be avoided. Further, any substance, at some level of concentration, can interfere with enzyme activity through non-specific or physical means. For example, some substances, at high enough concentrations, will denature proteins. The results could appear as if enzyme activity were inhibited, whereas in actuality, the results only reflect an unrealistically high concentration of the test material in the assay. Guidance by EPA on dose setting and data interpretation is necessary to avoid such 'false positives.'

The Council appreciates this opportunity to provide early input on matters related to the EDMVS. We look forward to working further with EPA and other interested parties on the validation of EPA's EDSP. Please don't hesitate to call me (703-741-5210) if you have questions.

Sincerely,

Original Signed By

Richard A. Becker, Ph.D., DABT
Public Health Team

cc: Jim Kariya, Office of Science Policy and Coordination, EPA
Gary Timm, Office of Science Policy and Coordination, EPA
Jane Smith, Office of Science Policy and Coordination, EPA